Brief Review

Nonadrenergic Neural Vasodilator Mechanisms

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Vasomotor influences mediated through peripheral nerves were first demonstrated by Claude Bernarde. After many decades of experimentation, few doubt that the sympathetic outflow mediated through norepinephrine and epinephrine is the major physiological vasomotor mechanism. However, nonadrenergic nerves supply most regional vascular beds, and their possible influence must be taken seriously.

In this review, we survey the evidence for functional nonadrenergic dilator innervation of regional vascular beds in common laboratory animals and man with particular emphasis on the criteria for the establishment of a neuroeffector transmitter. Simply stated, these criteria require that a putative transmitter substance 1) be present in the nerve terminal (ideally the synthetic machinery should be demonstrated in the neurone, but this is rarely satisfied); 2) be released in response to normal dromic propagated activity in the axon; 3) exert an action on the postsynaptic membrane of the effector cells when released during nerve activity that is similar to that observed for the nerve-mediated response, and finally, 5) effective pathways for the disposition of the putative transmitter should be present in the region of the synapse. Results derived from in vitro isolated blood vessel techniques, including biochemistry and histochemistry, are given prime consideration since data gained through these approaches are most relevant to the demands of these criteria. Data derived from in vivo studies by necessity are less rigorous. However, this approach does frequently give direction to more detailed investigation; the results of in vivo studies when they provide pivotal evidence are included.

It has been long held that the postganglionic sympathetic transmitter is a catecholamine, and the transmitter at the postganglionic parasympathetic terminal is acetylcholine. In practice, any peripheral postganglionic system that is nonadrenergic is placed in the convenient category of parasympathetic and generally considered to be cholinergic until proved otherwise. A number of plausible explanations or rationalizations have been made that allow this proposition to be retained even when evidence does not seem obviously consistent. As is quite evident from this review, there are a number of possible alternative transmitters. In many instances, the classic dogma has been challenged, so that acetylcholine transmission can no longer be assumed on the basis of one or two types of observations, and classic criteria must be satisfied. The parasympathetic system may be seen to contain a variety of efferent systems.

In vitro experimentation is desirable for a number of reasons. It allows examination of blood vessels in isolation from the tissues that they supply, which in many cases receive an innervation identical to that which distributes to the blood vessels of interest. A simplified in vitro system allows the various techniques used to establish a particular substance as a transmitter—electrical field stimulation of perivascular nerves, measurements of transmitter release, action, disposition, etc.—to be more easily executed and the results to be interpreted with less ambiguity. Current in vitro procedures allow observations to be made in vessels smaller than 100 μM. However, there are limitations; the vessel is likely to be damaged by dissection, possible local influences (such as axon reflexes) are excluded as are the modulating effects of substances released from surrounding cells, and there is interruption of the perivascular circulation. Finally, the temporal pattern of nerve activity, which seems to be important in the response, cannot be duplicated by experimental stimulation of the perivascular plexus.

Of the many problems in experimentation in this area, those related to the use and misuse of antagonist drugs often seem to be the least recognized or appreciated. Pharmacological specificity is relative and, even with the best blocker, is never absolute. No antagonist is without its side or additional effects. The convenient "blocker name" bestowed on a drug does not mean that this is the only activity that it will exercise, and the corollary is also true: When used as an antagonist, exerting a particular effect, this is not necessarily the consequences of its commonly held blocking action. Affinity for the same receptor in different tissues is not necessarily a constant. Sometimes there is evidence suggestive of transmission by several substances in a particular blood vessel, and in these circumstances a
Table 1. Summary of Evidence That Various Substances Are Neural Vasodilator Transmitters

<table>
<thead>
<tr>
<th>Substance</th>
<th>Best site</th>
<th>Present in nerves</th>
<th>Released from nerves</th>
<th>Postganglionic-exogenous effect</th>
<th>Specific receptors</th>
<th>Pharmacological potentiation</th>
<th>Pharmacological blockade</th>
<th>Breakdown mechanisms</th>
<th>Physiological significance</th>
<th>Neuroanatomy</th>
<th>Other species</th>
<th>Purines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>Cephalic arteries of the cat</td>
<td>+ (Chemical)</td>
<td>+</td>
<td>Probably</td>
<td>+ (Pharmacological evidence)</td>
<td>+</td>
<td>+ (Highly selective antagonists)</td>
<td>Cholinesterase</td>
<td>Possibly temperature regulation; increased blood flow associated with increased function</td>
<td>Some information, relatively sparse</td>
<td>Marked species differences in some cholinergic-related mechanisms</td>
<td>Rabbit portal vein</td>
</tr>
<tr>
<td>VIP</td>
<td>Cephalic arteries of the cat</td>
<td>+ (Chemical)</td>
<td>+</td>
<td>+</td>
<td>+ (Ligand binding studies)</td>
<td>+</td>
<td>+ (Antibody effect)</td>
<td>Limited evidence</td>
<td>Possibly thermal regulation</td>
<td>Detailed in cat; some for rat and guinea pig</td>
<td>Most mammals</td>
<td>Canine kidney</td>
</tr>
<tr>
<td>Histamine</td>
<td>Gracilis muscle, paw of dog</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+ (Membrane-bound peptidases)</td>
<td>Hypothesized (baroreflex)</td>
<td>Unknown</td>
<td>Some available; not detailed</td>
<td>Dog, cat, rat, primates</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Canine kidney</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ (Specificity questionable)</td>
<td>+</td>
<td>Sparse</td>
<td>Sparse</td>
<td>Rat, rabbit, cat, human</td>
<td>+</td>
</tr>
<tr>
<td>Purines</td>
<td>Rabbit portal vein</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Limited</td>
<td>Cat, guinea pig, rat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

? = evidence equivocal/inconsistent; + = evidence supportative; and . . . = evidence not available.

causative role for putative transmitters can best be provided only by the use of specific antagonists. Thus, the availability of specific agents is crucial. To be persuasive, evidence must be sought to show that an antagonist is specific for the claimed action under the circumstances in which it is used.

In this review, the evidence that particular candidate substances have a neuro-effecter role is discussed in turn. The various transmitters considered and their possible interactions are indicated in Figure 1. The evidence that a particular substance is a neurotransmitter in the blood vessel where the evidence is most persuasive is shown in Table 1.

**Acetylcholine**

Endogenous acetylcholine tissue levels in blood vessels have not been commonly measured. Histochemical measurement of acetylcholinesterase is not a satisfactory substitution. Although this enzyme is seen where cholinergic nerves are known to occur, it is also found in association with sympathetic nerves. A high affinity neuronal choline uptake system and the presence of choline acetyltransferase (CHAT) are fairly reliable indexes of cholinergic nerve presence, although exceptions have been reported. Based on such criteria, cholinergic nerves have been reported in many arteries of the head of the cat, rabbit, and dog. Most extensive has been a study in the cat in which a system accompanying arteries to tissues of the head including the brain, face, eye, nose, mouth, and tongue have been reported. In the latter study, acetylcholine content was shown to parallel choline acetyltransferase activity in a series of cephalic arteries. A somewhat similar distribution is found in the cephalic circulation of the monkey (J.A. Bevan, unpublished observation). Only in the arterial tree from the head of the cat has cholinergic transmission been unequivocally established. Many cephalic arteries from a number of species are innervated by neurones containing two types of vesicles based on the appearance of potassium permanganate (K$_2$MnO$_4$) fixed tissue, and designated granular and agranular. The agranular type has been associated with dilator innervation and acetylcholine. However, Gibbins has argued that the nature of the transmitter cannot be inferred from conventional electron microscopy. Acetylcholine was associated, although not exclusively, with small, clear vesicles in subcellular fractions prepared from the cat submaxillary gland. Release of acetylcholine on stimulation of perivascular nerves has been demonstrated in the cat.
Serotonin | Substance P
---|---
None obvious | Cerebral arteries of the cat + | (RIA, immuno-histochemical)
None obvious | Liver and membrane-bound peptidase + | (Antidromic activity)
Unclear | Nociception — otherwise pathophysiological
Not specific | Limited selectivity | (Pharmacological evidence)
Limited (origins in raphe nuclei) | peripheral detailed in guinea pig and rat

FIGURE 1. Illustration indicating the putative transmitters considered in this review and the possible sites of interactions between the different neuronal systems. Key: NPY, neuropeptide Y; NE, norepinephrine; 5-HT, 5-hydroxytryptamine; VIP, vasoactive intestinal polypeptide; ACh, acetylcholine; EDRF, endothelial derived relaxing factor.

Acetylcholine dilates most arteries from most species. The repeatedly observed vasodilator effect of acetylcholine in vivo could not be confirmed in vitro until it was shown by Furchgott and Zawadzki that it depended on an intact endothelium. Estrada et al. have shown [3H]quinuclidinylbenzilate (QNB) binding sites on intracerebral vessels that do not have vascular smooth muscle. Acetylcholine appears to release dilator material through an action on muscarinic endothelial receptors, designated endothelium-derived relaxing factor(s) (EDRF), that enter the tunica media and cause smooth muscle relaxation. Possible physiological relevance of this mechanism arises from the observations of Parnavelas et al. who localized CHAT within vascular endothelial cells in the rat brain.

Mechanisms of cholinergic vasodilation that do not involve EDRF have recently been reported. In the feline posterior auricular and lingual artery (Bevan and Brayden, unpublished observation), inhibitory muscarinic receptors are present on vascular smooth muscle cells.

There is some debate as to whether acetylcholine released from the perivascular plexus can reach receptors in the vessel wall to cause vasodilation. Angus et al., studying the dog femoral artery in vivo, showed that topical acetylcholine could cause endothelium-dependent relaxation when applied in concentrations some 50–100 times greater than that required to elicit relaxation when applied on the inside surface. The implication of these experiments is not supported by those of Cohen et al. However, if inhibitory muscarinic receptors are present in the media, as seems to be the case for some cholinergically innervated arteries, the problem is obviated. Neurogenic cholinergic influences on veins appear to be constrictor. In addition to an action on postsynaptic muscarinic receptors, acetylcholine might modify sympathetic or other transmitter release from perivascular nerves at presynaptic muscarinic autoreceptors. Such an effect has been proposed by Van Hee and Vanhoutte for cholinergic innervation of dog gastric arteries where adrenergic neurotransmission excited by field stimulation is modified by concurrent vagal activation, an effect that is enhanced by anticholinesterases and blocked by atropine.

Bell studied the membrane potential changes associated with perivascular stimulation in the guinea pig uterine artery using intracellular electrodes and was unable to find changes in membrane potential associated with cholinergic transmission. Furthermore, high concentrations of acetylcholine did not influence membrane potential. However, a more recent study has revealed membrane hyperpolarizations that are associated with endothelial-cell independent cholinergic neurovasodilation in the rabbit lingual artery.

The availability of the relatively specific muscarinic antagonist atropine has provided a simple primary intervention useful to test for cholinergic vasodilation in a vast number and variety of tissues. However, even this drug is not specific to the muscarinic receptor in all tissues and has been reported to antagonize at serotonin- and α1-adrenoceptor sites.

Thus, the normal discretions that apply to the use of...
antagonists in general cannot be ignored with atropine whose action, particularly in the lower dose range, is generally taken as presumptive evidence of cholinergic transmission. Atropine-sensitive neurogenic vasodilation has been reported in many tissues and species. In vitro vascular tissues in which partial or complete atropine block of neurogenic vasodilation has been reported include the guinea pig uterine artery and many arteries of the head of the cat. Lee has reported that atropine has no effect on electrically elicited vasodilation in cat cerebral arteries.

In vivo or perfusion studies where atropine has been reported to influence neurogenic vasodilation are extensive and cannot be cited or discussed in this review. They include many tissues in the head of the cat, the brain, choroid plexus, lacrimal gland, upper and lower eyelids, skin of the face, salivary glands, ophthalmic choroid, iris, ciliary body, nictitating membrane, and in both the mucosa and muscle of the tongue. This distribution closely corroborates with in vitro assessments. In addition, cholinergic neurovasodilation has been reported in the circulations to the lung, heart, uterus, skeletal muscle of some species but not primates and in the kidneys, liver, colon and rectum, part of the penile erectile system, and the paw. Reported species differences in many of the above phenomena are remarkable.

Since cholinergic transmission is terminated by enzymatic degradation of acetylcholine, anticholinesterase action leading to potentiation of transmitter effect is also an essential, but by itself an insufficient, criteria of cholinergic vasodilation. Potentiation of neurovasodilation has been seen in vitro in arteries from the cat head and guinea pig uterus. In many instances in vivo, anticholinesterases have been shown to potentiate neurovasodilation. In some instances where atropine provided blockade, anticholinesterases did not potentiate for reasons unknown.

Knowledge of the neuroanatomy of the cholinergic vasodilator fiber systems is incomplete. In the vasculature of the head of the cat, the distribution of cholinergic nerves (assessed by the CHAT activity) and vasoactive intestinal peptide are not the same. There is inconsistency in findings concerning the pathways and origins of the cholinergic innervation of the cerebral circulation. The central organization of the cholinergic vasodilator outflow to striated muscle of carnivores is similar to that mediating noncholinergic vasodilation to this tissue in the primate and involves loci in the lateral hypothalamus, the perilemmisal region of the mesencephalon, and the region of the lateral spinothalamic tract in the medulla. They reach skeletal muscle through the sympathetic outflow.

Our discussion of the significance of cholinergic vasodilation is influenced by the confidence we have in our conclusion that acetylcholine is a transmitter at particular sites. In a number of vascular regions (but not in all species), acetylcholine and vasoactive intestinal peptide seem to be associated in nonsympathetic control. Lundberg has developed this concept for the cat exocrine gland vascular supply. These two substances are certainly closely associated in the perivascular nerves to arteries that supply many tissues of the head of the cat, but overlap of their distribution is not absolute. The overall problem of the consideration of the distribution of the two types of innervation has lead to the proposal that this outflow might be involved in thermoregulation, in particular, in maintenance of the constancy of the temperature of the brain (for discussion, see Gibbins et al). It would also seem reasonable to conclude that neurocholinergic mechanisms are necessary for the smooth functioning of many tissues during their parasympathetic activation, helping to relate changes in blood flow and function. Many examples of this might be inferred from the citations above for in vivo atropine effectiveness. Feigl has shown that cholinergic coronary dilation can be elicited reflexly in the dog. Cholinergic vasodilation of resistance vessels of skeletal muscle seems to be the mechanism of anticipatory increase of blood flow prior to exercise.

### Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP), a highly basic 28 amino acid polypeptide, was first isolated from porcine small intestine and named because of its potent hypotensive effect following systemic injection. Sequence homologies place it in the secretin-glucagon family of peptides.

Following the purification and subsequent development of specific antibodies, VIP-immunoreactivity was demonstrated not only in the gut but also within nerves supplying many other organ systems. Nerve terminals are most intimately associated with smooth muscle and glandular and mucosal cells (see Fahrenkrug and Hokfelt et al). VIP-containing nerves have now been described in the central nervous system, gastrointestinal tract, genitourinary tract, exocrine glands, lung, eyes, heart, kidneys, and skeletal muscle of many species. Perivascular VIP-immunoreactive nerve fibers, restricted to the adventito-medial border, are present in all of these tissues and atropine-resistant neurogenic vasodilation has been reported in many of them, including the brain, nasal mucosa, tongue, eye, salivary glands, and gastrointestinal tract. Localization of VIP within large, dense core vesicles in feline salivary gland and cerebral artery perivascular axons and nerve terminals supports the concept of a specific transmitter role for VIP.

VIP-immunoreactive (VIP-IR) nerve cell bodies are widely distributed and in most cases are found within, or in close proximity to, innervated tissues. In the cat, each of the major cranial parasympathetic ganglia, with the exception of the ciliary ganglia, contains VIP-IR cell bodies. Greater than 95% of the cell bodies in the sphenopalatine ganglion are VIP-IR, and these cells are the source of VIP-IR perivascular nerves that supply the feline nasal mucosa and ocular structures. The sphenopalatine ganglion is also the source of some of the VIP-IR perivascular axons in the cerebral circulation of the rat. Ganglia of the sympathetic
nervous system also contain VIP-IR cell bodies. Intrinsic VIP-IR cells, which may supply blood vessels, are found within, among other tissues, the gut, bladder, bronchi, pancreas, thyroid, and heart. VIP is synthesized in the neuronal cell body, packaged into secretory granules, and carried by axonal transport to the nerve terminals. A large molecular weight precursor of VIP has been identified, but the mechanisms of cleavage of the precursor to form smaller active fragments that include VIP and related peptides (peptide HI or peptide HM in humans) have not been characterized.

VIP is released during transmural nerve stimulation of isolated feline lingual and cerebral artery segments, which show a significant atropine-resistant neurogenic vasodilation. Stimulation of the autonomic nerves that supply the feline salivary glands, bovine and porcine gastrointestinal tract, and feline nasal mucosa results in vasodilation and overflow of VIP. Much of this VIP may come from perivascular nerves, although it is probably released from nonvascular nerves as well. VIP may be degraded by peptidases located near the site of release although such enzymes have not been isolated or characterized. Pathways for reuptake of peptides have not been demonstrated. The only other mechanism for termination of the response to endogenously released VIP is removal through the circulation and subsequent degradation in the liver.

Nanomolar to micromolar amounts of exogenous VIP induce vasodilation which equals or exceeds that caused by nerve stimulation in feline cerebral, salivary gland, lingual, and porcine splanchnic arteries. In most cases, VIP appears to act directly on vascular smooth muscle rather than indirectly through endothelial cells or by the release of a second factor. Although indomethacin did reduce the response of some feline cerebral arteries to exogenous VIP, suggesting the involvement of a prostaglandin, this has not been confirmed.

Receptors for VIP in bovine cerebral arteries have been characterized using ligand binding techniques. VIP is significantly more potent than several related peptides (PHI, PHM, rat growth-hormone releasing factor) both in displacing labelled VIP from binding sites and in causing vasodilation of cerebral arteries. VIP stimulates adenylate cyclase in rat cerebral microvessels as well as in nonvascular tissues. Elevations of cyclic AMP in response to VIP occur in the rabbit mesenteric artery.

Specific VIP antagonists have not yet been developed. However, VIP antibody has been used with success in several instances to inhibit nonsympathetic, noncholinergic neurogenic vasodilation. In the feline lingual and middle cerebral arteries in vitro, VIP antiserum but not substance P antiserum inhibits the dilator response to nerve stimulation as well as that induced by exogenous VIP. VIP antibody also inhibits dilator responses to autonomic nerve stimulation in the feline submandibular salivary gland and in the feline extracranial cephalic circulation in vivo. Avian pancreatic polypeptide has been reported to be an inhibitor of VIP-mediated dilator responses in vivo, but this observation could not be confirmed in a number of other arteries using in vitro techniques (J.E. Brayden and J.A. Bevan, unpublished observation).

In the cat salivary glands, cholinergically mediated secretory events appear to be coupled with vasodilation mediated by VIP. It is not clear why two dilator transmitters are required. It may be that enhanced blood flow is required during periods of increased salivation, and VIP is probably the responsible vasodilator substance. In the porcine gut, a hyperemic period, apparently mediated by VIP, coincides with mechanical stimulation of the intestinal lumen. In the feline cephalic circulation, a role for cholinergic and VIP-IR nerves in thermoregulation has been postulated. VIP is also present in perivascular nerves that supply intraparenchymal cerebral arteries of the rat. In addition to causing dilation of cerebral arteries, VIP can depolarize and excite various types of cerebral neurons and mobilize energy stores in cerebral tissue. These observations suggest that VIP plays a role in the increase of blood flow associated with cortical activity.

In summary, VIP may be a transmitter that via a nonsympathetic autonomic outflow mediates vasodilation in many vascular beds. In fact, all essential criteria for vasodilator neurotransmitter status have been demonstrated convincingly for VIP using well-controlled in vitro methodology. This finding is unique for VIP in comparison to all other nonadrenergic, noncholinergic putative vasodilator transmitters and places VIP alongside acetylcholine as one of the best established dilator transmitters.

**Histamine**

Beck and Beck and Brody showed that reflex dilation in limb muscle is not merely due to withdrawal of sympathetic constrictor tone but includes an active neurodilator component, which could be attenuated by an antihistamine. These initial observations have led to a great number of attempts to characterize a possible histaminergic outflow to blood vessels.

Histamine has been found in high concentrations in sympathetic and, to a lesser extent, parasympathetic nerves of dogs and monkeys (10–20 μg/gm). It is not depleted by reserpine or 48/80, a mast cell depletor. The observation that removal of adventitia does not significantly reduce blood vessel histamine suggests that this amine is not concentrated in nerve terminals. This conclusion is consistent with attempts to demonstrate histamine within perivascular nerves using a histofluorescence technique. Mast cells are found around some blood vessels of some species, particularly rodents and cattle. Arterial histamine content does not change after chronic sympathectomy.

Measurements of histamine content have been restricted to larger vessels. This is unfortunate, since if histamine is a dilator transmitter or modulator of transmission, it would only be effective on vessels with tone, a feature that is usually not found in most larger...
arteries and veins. In addition, responses of larger vessels to electrical field stimulation generally are not influenced by antihistamines.

Histamine is generally higher in veins than in arteries. It is synthesized in the vessel wall and is actively taken up by vascular smooth muscle. No method of blocking uptake or of separating specific and nonspecific binding other than low temperature has been documented. The amine is metabolized by histamine-0-methyl-transferase and, more importantly, diamine-oxidase. In the rat, however, imidazoleacetic acid and methyl histamine formation is an important route.

Histamine release from isolated blood vessels has not been demonstrated. The in vivo observation that, after muscle loading with labelled histamine or its precursor, reflex activation is associated with release of labelled material is subject to a number of interpretations.

There are ample possible mechanisms whereby histamine may reduce vascular tone. H1- and H2-receptors are present on sympathetic nerve terminals, and histamine acting through H2-receptors reduces norepinephrine efflux from sympathetic nerves on activation. It also causes dilation through H2-receptors on the vascular smooth muscle cell and on the endothelium. The rat aorta endothelium contains an inhibitory H1-mechanism. H2-receptors are considered to be dominant in mast cells. Only H1-receptors are found in veins. H1-blockers frequently only attenuate vasoconstriction. There is a report that both H1- and H2-receptors are involved in reflex vasoconstriction that occurs to sympathetic nerve stimulation after adrenergic neuronal blockade and at the cessation of sympathetic nerve stimulation. Histamine has been shown to both potentiate and inhibit ongoing sympathetic activity.

Vasodilator responses after exposure to atropine can be elicited by increasing the carotid sinus pressure and stimulation of the carotid sinus nerve, electrical stimulation of the medulla, midbrain, and hypothalamus; the spinal ventral roots; the sympathetic outflow; and by the ganglionic stimulating drug DMPP. The dilator effect is blocked by a ganglionic blocking agent. Invariably, the dilator response elicited either before or after atropine is attenuated by an antihistamine, particularly an H1-blocker. Many investigators have claimed that the antihistamines at the doses used were selective and specific.

Vasodilation, which can be mediated by histamine, has been shown to occur in the gracilis and gastrocnemius muscle and at low frequencies of stimulation in the paw. Histamine has been implicated as a vasodilator in the ear of the dog and in muscle of the dog, cat, and rat, and in primates. Histamine has also been implicated in the dilation caused by postganglionic sympathetic nerve stimulation in vivo. However, the distribution of this dilator influence throughout the arterial bed has not been systematically explored.

There is a convincing association between sympathetic outflow activity, vasodilation, and the blocking action of antihistamines. Histamine is appropriately present in many cells in the vessel wall. The precise interrelation between these and many other observations is far from established. The weakest link is the action of the antihistamine. It is, however, relevant that adrenergic responses of the heart on sympathetic nerve stimulation are modified by an H2-antagonist. Gross et al suggest that this represents a "physiological role" or at least evidence that histamine is involved in a physiological mechanism in the normal heart. However, evidence of this type for a limited number of vascular beds has been in existence for more than two decades. Taken together, the idea that histamine is in some way or another involved in normal adrenergic neuromechanisms is persuasive.

This dilator system has been implicated in "playing dead," emotional syncope, and is quantitatively changed in renal and DOCA hypertension in the rat. Since it has been seen consistently after carotid sinus stimulation, it is probably part of the normal baroreceptor reflex. It is not involved in the dilation of reactive hyperemia.

**Dopamine**

Dopamine is a precursor catecholamine in the synthesis of norepinephrine (NE) and is found in appreciable amounts in all adrenergic nerves. However, outside of the central nervous system, it has been difficult to establish a distinct physiological role for dopamine. Nevertheless, there is evidence to suggest that it is a vasodilator transmitter in nerves that distribute to the kidney, and possibly the mesentery and the canine paw pad circulation.

Although it has been suggested that dopamine histo-fluorescence and patterns of innervation are sufficiently different from those of NE to allow discrimination of nerves containing these two substances, the reliability of such analyses is questionable. Using an accurate microspectrofluorometric method, Dinerstein et al found that renal cortical and juxtaglomerular perivascular nerves contain large amounts of dopamine and little or no NE, whereas renal medullary arterial innervation is primarily nonadrenergic.

Direct biochemical measurement of tissue dopamine levels provides only inconclusive evidence due, as mentioned above, to the presence of dopamine in noradrenergic systems. One approach has been to measure dopamine/NE ratios in different tissues, the rationale being that such ratios should be elevated in tissues containing these two substances, the reliability of such analyses is questionable. Using an accurate microspectrofluorometric method, Dinerstein et al found that renal cortical and juxtaglomerular perivascular nerves contain large amounts of dopamine and little or no NE, whereas renal medullary arterial innervation is primarily nonadrenergic.

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The possible origins of the dopaminergic outflow to the kidney and hind limb have been demonstrated in
combined studies of catecholamine and dopamine-β-hydroxylase fluorescence in canine sympathetic ganglia.156 Dopamine-β-hydroxylase should be absent from dopaminergic neurons. Some cells within para-vertebral sympathetic ganglia (T11-L2 and L5-S2) that supply the vasodilator outflow to the kidney and hind paw exhibit catecholamine fluorescence but contain no detectable dopamine-β-hydroxylase immunohisto-fluorescence.

Release of dopamine into the venous outflow of the rat and dog kidney occurs on stimulation of the renal nerves.157 However, the amount of dopamine released could actually be discharged from adrenergic nerves during such stimulation.155

Exogenous dopamine dilates a number of different arteries and vascular beds in vitro including the canine and rat renal,158 canine and rabbit mesenteric,159,160 canine coronary,161,162 canine, feline, and human cerebral,163,164 and small (<1.0 mm o.d.) canine femoral arteries.165 Following α-adrenoceptor blockade, and in many cases after addition of a constrictor agent, dose-related relaxations to dopamine (10⁻⁷–10⁻¹⁴ M), not affected by β-adrenoceptor, cholinergic, or histamine antagonists but inhibited specifically by dopamine antagonists, can be demonstrated. Dopamine analogues having dopamine agonist activity have been developed, and compounds such as haloperidol, sulpiride and bulbocapnine as well as a number of newer agents have been recognized as specific dopamine antagonists. A specific dopamine receptor (DA₁) that mediates vasodilator responses and is located on vascular smooth muscle cells has been confirmed by many studies.166–168

A second dopamine receptor (DA₂) is an adrenergic inhibitory presynaptic autoreceptor.169,170 It is frequently found in vascular beds that lack postsynaptic dopamine (DA₂) receptors and probably contributes to the hypotensive effect of systemically administered dopamine by reducing sympathetic adrenergic activity.

Dilator responses of renal and paw pad arteries in vivo to exogenous dopamine are similar to those observed following stimulation of descending autonomic pathways in the dog.130,171 and both are inhibited in a dose-dependent manner by haloperidol and ergometrine. Central infusions of ouabain produce increases in renal blood flow that are abolished by sulpiride,172 a specific DA₁ antagonist. Sulpiride also inhibits the dilator effect of renal nerve stimulation in the rat,173 and haloperidol inhibits the dilator response to stimulation of the splanchnic nerve or of perivascular nerves in canine mesenteric arteries.160 Infusion of sulpiride without other intervention causes reduction of renal blood flow in rats, suggesting a tonic effect of dopaminergic nerves.173

Mechanisms of termination of the effects of neurogenic dopamine have not been fully characterized. Uptake of dopamine into sympathetic nerves readily occurs,174,175 and monoamine oxidase and catechol-0-methyl-transferase enzymes can convert dopamine to its metabolite products, homovanillic acid and 3, 3-dihydroxyphenylacetic acid.176,177 Thus, the major routes of inactivation of dopamine are probably identical to those for NE.

The physiological significance of the dopaminergic outflow is little understood. In the kidney, these nerves may regulate blood flow to cortical structures178 as well as possibly influence renin secretion and sodium and water excretion. Speculation regarding possible neurovasodilator function in other tissues is premature and must await better evidence for a neurovasodilator role.

Purines

The vasoactivity of ATP and compounds related to it has been known since the early studies of Drury and Szent-Gyorgyi179 and confirmed in many blood vessels from many species. ATP and its degradation products are potent dilator and constrictor agents with complex actions on the vasculature. Evidence that might be considered strongly supportive of a role of purines in neurotransmission and neuromodulation in the circulation is relatively recent. Prior to this, the site(s) for which experimental data was most convincing (and criticism most vociferous) was the inhibitory neurotransmission mechanism in the enteric nervous system. The crucial problem is that the purines, particularly ATP, are involved in a profusion of essential biochemical processes within cells, including transmitter release and possibly modulation, and, for this reason, it is exceedingly difficult to sort out such closely related events.

The term "purinergic" was first introduced and to a great extent subsequently explored and elaborated by Burnstock.180 The purinergic hypothesis, developed mainly in nonvascular tissues, is that ATP is released from nonadrenergic nerves to act directly on constrictor effector cells and is also a cotransmitter modulating the contractile effects of norepinephrine. Both these actions could then lead to vasodilatation, although recent evidence suggests that vasocostriction mediated by purines may occur in some tissues.181 Although an in vivo study potentially implicates the purines as vasodilator transmitters in a skeletal muscle bed of the rabbit,182 the majority of evidence for purinergic transmission is derived from in vitro or simplified tissue systems.

ATP and the enzymes that synthesize it are present in all cells, including adrenergic neurons. It seems to be stored in the vesicles with norepinephrine in a ratio of 1:7–12, 183 although this is not invariably the case.184 In the rabbit portal vein, there is evidence for purinergic neurons.185 Quinacrine histofluorescence, considered indicative of purinergic nerves, has been observed around many blood vessels from rabbit, guinea pig, and rat. In the rabbit portal vein, where the most detailed corroborative functional studies have been undertaken, single and also bundles of varicose axons are present at the adventitio-medial junction and also between the longitudinal and circular muscle layers. As the fluorescence is resistant to 6-hydroxydopamine, it is thought to originate from nonadrenergic neurons. A
few intramural ganglia (4–6/1.0–1.5 cm length of tissue) also resistant to chemical sympathectomy are reported. This pattern is not seen in the portal veins from the rat and guinea pig.  

Release of H purines has been observed after incubation in 3H-adenosine of the rat and rabbit but not the guinea pig portal vein. It is incorporated into 3H-ATP.  

Labelled purines are also released from the rabbit ear artery and thoracic aorta, from a preparation of only aortic adventitial tissue\textsuperscript{182} and from perivascular nerves supplying the dog cerebral arteries.  

Re-lease occurred after guanethidine and 6-hydroxydopamine treatment and to a smaller degree after cervical sympathectomy. Since the uptake of \textsuperscript{3}H-adenosine studied by Burnstock and others was unchanged by chronic sympathetic denervation, we might presume that the label was taken up into nonsympathetic nerves. There is abundant evidence for nonadrenergic, non-cholinergic innervation of cerebral arteries for which a number of neurotransmitters have been suggested.  

ATP may be a neuromodulator, activating presynaptic autoinhibitory receptors influencing norepinephrine and possibly its own release.  

\textsuperscript{3}H-NE efflux in response to field stimulation, but not tyramine, is reduced by adenosine and related nucleotides in blood vessels of the dog, rabbit, and rat.  

The presynaptic effect of ATP has a threshold of 0.1 \mu M and does not, like its postsynaptic effect, show tachyphylaxis. Its metabolites are inactive.  

The presynaptic purinergic receptor does not conveniently fall into either P\textsubscript{1} or P\textsubscript{2} categories\textsuperscript{192} (see below). It has been proposed that there are purinergic receptors (P\textsubscript{1}) located on the adrenergic nerve terminal through which adenosine causes an inhibition of ongoing norepinephrine release blocked by theophylline. There is also evidence for a presynaptic (P\textsubscript{2}) receptor that positively influences the magnitude of transmitter release. The role of the latter receptor, however, is far from established.  

The debate as to whether on field stimulation purines are derived from postsynaptic structures to act presynaptically has not been engaged in the vascular field. However, in nonvascular tissue, the argument that they predominantly originate from neural tissues is persuasive\textsuperscript{180} and agrees with the most detailed studies carried out so far in vascular tissues.  

Once released, there is some presumptive evidence that the postsynaptic events and effector changes are similar to those caused by exogenous ATP.  

The following receptor classification has been proposed by Burnstock.\textsuperscript{186} There is a purinergic, (P\textsubscript{1}) receptor with an agonist order of potency-adenosine > AMP > ADP > ATP and a purinergic, (P\textsubscript{2}) receptor with a reverse order. Antagonists of P\textsubscript{1} include methylxanthine and of P\textsubscript{2} quinidine, phenoltalamine, and antazoline, but these antagonists are of low specificity and potency. Since this classification was originated, some new compounds have been incorporated into the classification scheme.  

Based on a detailed study of the rat femoral artery,\textsuperscript{197} the following distribution of receptors has been proposed in vascular smooth muscle. Relaxation to ATP occurs mediated via P\textsubscript{2} receptors on the endothelium. In some blood vessels, however, adenosine seems to be quite effective at this site. P\textsubscript{2} receptors on the vascular smooth muscle cells also mediate dilation, but in addition, these cells contain P\textsubscript{1} receptors which mediate contraction. α-β-Methylene ATP, a more stable analog commonly used to help unravel purine mechanisms, is more potent on P\textsubscript{2} than P\textsubscript{1} in the rat femoral artery. More experiments need to be carried out in a variety of vessels from many species before any generalization can be hazarded. There are recent arguments for two further purinergic receptor subtypes—A\textsubscript{1} and A\textsubscript{2} —for adenosine. A\textsubscript{1} is associated with inhibition and A\textsubscript{2} with stimulation of adenylyl cyclase. A\textsubscript{1} receptors have been identified in the adrenergic nerves of the rabbit portal vein.  

Extracellular ATP is degraded at the surface of both vascular endothelial and smooth muscle cells by an ectoenzyme system consisting of nucleoside triphosphatase, nucleoside diphosphatase, and 5'-nucleotide. The resultant adenosine, which has effects on vascular tone, is inactivated by adenosinedeaminase.  

For adenosine disposition, the major route is uptake into cells and subsequent phosphorylation through extracellular adenosine, an important initiator of reactive hyperemia.  

It is one thing to show that a transmitter system is present; it is another to show that it has a physiological role. In the rabbit and rat portal vein, prejunctional inhibitory receptors are present, and their activation leads to inhibition of release of ATP, both from adrenergic and nonadrenergic nerves. In the guinea pig, no preganglionic receptors are present and no ATP-containing nerves are amongst its innervation. This is considered supportive of a physiological role for neurally-released ATP. Certainly, the coincidence of the findings is provoking.  

Inhibitors of adenosine uptake and deamination (diazepam and erythro-9-[2-hydroxy-3-nonyl] adenine) reduced the overflow of \textsuperscript{3}H-NE and contraction during nerve stimulation from the rat portal vein.  

Phe-nolphthalein had the same effect on the rat portal vein\textsuperscript{189} but was ineffective in the dog basilar artery.  

This feedback inhibition may be diminished in the mesenteric vasculature of the spontaneously hypertensive rat.  

Shimada and Stitt\textsuperscript{182} demonstrated vasodilatation in the rabbit skeletal muscle vasculature in response to hypothalamic stimulation mediated through sympathetic nerves that was insensitive to blockers of many putative transmitters. The authors’ use of antagonists did not unequivocally clarify the transmitter involved.  

In summary, the best evidence for vascular neurotransmission by purines is from studies of the rabbit portal vein. There is preliminary data from studies of several arteries suggesting a neuromodulator role; however, there are uncertainties that remain to be elucidated.  

5-Hydroxytryptamine  

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under some conditions, particularly when vascular tone is high, this indolamine causes vascular relaxation. It should, therefore, be considered as a possible dilator transmitter. On close inspection, however, few of the criteria for vasodilator transmitter status are upheld.

Chan-Palay, using autoradiographic techniques, has observed 5-HT within cerebral artery perivascular nerves in monkeys and rats, and this finding has been confirmed by others in cats, rabbits, mice, guinea-pigs, and man using immunohistochemical and biochemical techniques. Pial and intraparenchymal blood vessels and the choroid plexus are innervated by nerves containing 5-HT, and the source of many of these appears to be the raphe nuclei of the mid-brain. The human fetal mesenteric circulation also receives 5-HT immunoreactive nerve fibers. Metabolic pathways for synthesis and degradation of 5-HT appear to be present in such nerves. However, there is little direct evidence that these nerves have a vasodilator role.

Evidence for nerve-evoked 5-HT-mediated vasodilator responses is limited to a few studies of intestinal vasodilator mechanisms. Close intraarterial injection of 5-HT causes intestinal vasodilation in the cat. Dihydroergotamine, which includes 5-HT antagonism amongst its actions, inhibits these responses. Enhanced release of 5-HT into intestinal venous blood occurs during electrical stimulation. However, 5-HT probably does not act directly on the vascular smooth muscle receptors since tetrodotoxin blocks the dilator action of exogenous 5-HT. Its effect is most likely mediated via an interneuronal pathway, probably releasing 5-HT from enterochromaffin cells, a non-neuronal source.

Intra-arterial injection of 5-HT causes dilation of the external carotid bed of dogs and baboons, as well as the canine coronary, splanchic, forelimb, and skeletal muscle beds. In the carotid and forelimb circulations, the dilator effect occurs predominantly in arterioles. In some or all of these studies, dilation may result from a presynaptic inhibitory effect of 5-HT on norepinephrine release resulting in a diminution of vascular tone. Direct microapplication of 5-HT to feline cerebral arteries in situ before or after chronic sympathectomy dilates those that have diameters of less than 70 µM. It constrains the larger arteries. Dilation only occurs when arterial pressure is normal or elevated; it is decreased or reversed during hypotension. Since propranolol inhibits this dilator response, its postsynaptic mechanism is problematical, and most likely β-adrenoceptors or a very similar receptor are involved.

In vitro, 5-HT causes dose-dependent relaxation of preconstricted feline middle cerebral, lingual, and external maxillary arteries and human cerebral and superficial temporal arteries (300–500 µm in diameter) provided that 5-HT constrictor effects have been blocked by phenoxybenzamine. In isolated, contracted cat saphenous and goat pulmonary veins, 5-HT causes a dose-dependent relaxation.

Little additional information is available regarding the neurovasodilator transmitter status of 5-HT or the physiological function it may subserve. Recently, it has been observed that dilator responses to exogenous 5-HT in canine and porcine coronary arteries and porcine renal and mesenteric arteries are mediated via the endothelium. Therefore, until evidence is presented that indicates a direct dilator action of 5-HT on arterial smooth muscle cells or that endothelial cells are innervated by 5-HT-containing nerves, the results of most of the experiments described above provide only meager support for a neurodilator role for 5-HT.

**Substance P**

In 1983, Lembeck proposed a role for substance P (SP) as a neurotransmitter in the central and peripheral nervous systems. There is now a compelling and persuasive body of evidence that it is at least a central neurotransmitter or neuromodulator. Its presence in the wall of many blood vessels (see below), combined with the recognition of its exceptional vasodilator activity, raises the possibility that it is a physiological neurovasodilator.

The presence of SP in the wall of blood vessels, both arteries and veins, has been demonstrated in many species. Particularly detailed studies have been made of the SP innervation of the middle cerebral artery of the cat, the entire arterial tree of the guinea-pig and the rat. SP fluorescent nerve networks are present in the adventitia and the adventitio-medial junction, a pattern commonly seen with vaso-motor nerves. In the guinea-pig, this plexus is most dense in the large central arteries and veins, and density diminishes peripherally along the ramifications of the arterial and venous trees. Immunocytochemical evidence exists for SP-containing cell bodies in many parasympathetic and sympathetic ganglia. The inferior mesenteric and celiac ganglia show the highest density. There are also SP-containing cell bodies in the carotid body, the carotid sinus nerve, and in sensory sympathetic ganglia and petrosal ganglion cells. SP fibers are present in many peripheral nerves, including the vagus, where axoplasmic flow is centripetal in direction. Free nerve terminals are present around blood vessels. All evidence, including that from retrograde tracing, immunohistochemistry, and capsaicin treatment, is consistent with the conclusion that SP is synthesized in sensory ganglia and is distributed to nerve terminals by fast axonal transport. SP levels are uninfluenced by sympathectomy. Treatment with capsaicin results in the loss of SP immunoreactivity from the blood vessels of the guinea-pig, with the exception of arteries supplying the distal intestine, and the cerebral vessels from cats and rabbits. Some findings are not always quite so clear-cut. Unilateral section of the trigeminal ganglia decreases SP levels in the cat pia arachnoid and accompanying blood vessels by just over 50%. The source of the remaining levels of SP is uncertain.

SP can be released by antidromic stimulation of presumably sensory neurons, an effect that is associat-
ed with vasodilation. This effect in the dental pulp blood vessels of the cat was inhibited by somatostatin. SP analogs inhibit the vasodilation induced by stimulation of the distal end of the saphenous nerve in the rat as well as by the infusion of SP. There is some evidence that stimulation of presumed sensory nerves leads to vasodilation influenced in part by atrpine and antihistamines. The possibility that the former effect is associated with concurrent stimulation of postganglionic cholinoergic efferent neurons and the latter with mast cells must always be borne in mind. There is no documented evidence of orthodromic SP release under physiological circumstance in the circulation. SP may play a role in axo-axonic mechanisms and in reactive hyperemia.

Most reports describe SP as a potent and effective vasodilator both in vivo and in vitro. However, the postynaptic effect of SP in a number of instances is not consistent with a neurovasodilator role. Repeated electrical intramural nerve activation can usually result in fairly consistent vasodilation. However, marked tachyphylaxis of effect of exogenous SP has been described in the cat, dog, and in all vessels tested by Furchgott. Furthermore, constriction of blood vessels and absence of effect have been reported. SP effects on arteries are generally endothelium-dependent; in fact, SP is the most potent endothelium-dependent relaxing agent known. This characteristic contrasts with dilator nerve activation.

By contrast, SP effects on veins are endothelium-independent. In fact, in vivo infusion of SP does not affect capacitance vessels. This lack of a clearcut association of neurovasodilation and SP is exemplified further by the lack of correlation between SP levels and neurovasodilation in a series of cephalic arteries of the cat and particularly in the radial artery, which contains high concentrations of SP but exhibits no neurovasodilation. Barja et al. have remarked concerning the striking lack of correlation between the presence of SP fibers and the vasodilator action of SP. It should be borne in mind, however, that the effects of SP are complicated by its histamine-liberating capacity (see "Histamine").

Effector cells probably contain three types of SP receptors, studied by Regoli et al. This recent, very comprehensive series of studies reveals differences between receptors on vascular and nonvascular tissue and also between those on different blood vessels from the same species. They comment that there are many interfering factors in their studies and that different cellular systems are very unpredictable. A number of antagonists are available, but they have relatively low selectivity. These include a baclofen, β-(4-chlorophenyl)-GABA, and a number of SP analogs of which [D-Arg1, D-Pro2, D-Trp7,9, Leu11] SP is the most potent. [D-Pro2, D-Trp7,9] SP shifted the dilator response curve of cat pial arteries to the right. However, its specificity in this vessel was not demonstrated.

In vivo infused SP is degraded mainly in the liver. Membrane-based peptidases are most likely to influence SP levels in the synapse. Lee has identified and purified a neutral metallo-endopeptidase with a similar distribution to membrane-bound SP.

Numerous observations link SP with nociception, specifically its localization to sensory-related systems, electrophysiological evidence linking it to pain fibers, and the effects of capsaicin. A persuasive series of studies strongly suggests its involvement in vascular headaches. Antidromic release seems to be associated with extravasation—increased vascular permeability and disruption of the blood-brain barrier. A role as a causal physiological neurotransmitter has not been formally proposed. No known neurovasodilator effects parallel the distribution of SP in the circulation.

Other Putative Transmitters

Several putative neurotransmitters, some of which have vasodilator activity, have been discovered in recent years. Some of these compounds are peptides, and a brief discussion of the properties of three of them follows. The reader is referred to recent reviews for a more detailed discussion pertaining to peptide neurotransmission.

**Peptide HI**

Peptide HI (PHI) was first isolated from porcine gastrointestinal tissue by Tatamoto and Mutt. It is a member of the secretin-glucagon family of peptides and has nearly 50% sequence homology with VIP. A peptide nearly identical to PHI, peptide HM (PHM), has been isolated from human neuroblastoma cells (VIPomas) and is thought to be the human equivalent of PHI. PHM and VIP are contained within a common precursor peptide, prepro-VIP.

Coexistence of PHI and VIP has been established using histochemical techniques in perivascular nerves that supply feline cerebral arteries and in pulmonary blood vessels. The overall distribution of nerves containing PHI is nearly identical to the distribution of VIP-IR nerves in the feline cerebral circulation. Like VIP, PHI is a vasodilator substance (endothelial-cell independent); however, although equally effective, it is 20–30 x less potent than VIP. Other transmitter criteria for PHI or PHM have not been reported. The physiological significance of their colocalization may reflect a previously unrecognized need for additional, subtle mechanisms of vascular control.

**Calcitonin Gene-Related Peptide**

Calcitonin gene-related peptide (CGRP) was first isolated in 1983 by Rosenfeld et al. and it has since been observed within perivascular nerves. The existence of this peptide was predicted based on nucleotide sequence analysis of the calcitonin gene; selective synthesis of either CGRP or calcitonin is tissue specific.

Periartrial nerves containing CGRP have been observed in the cerebral circulation of rats, guinea pigs, and humans in the heart, lung, and gastroin-
testinal tract of the rat and guinea pig in the rat kidney and limb circulations,' and in the rat tongue. CGRP coexists with substance P in cerebral perivascular nerves in the rat and guinea pig, and most of these nerve fibers originate in the trigeminal ganglion. Coexistence of CGRP and substance P has also been noted in spinal sensory neurons of the rat. Capsaicin treatment of neonatal rats depletes both substance P and CGRP in perivascular nerves that supply the brain, but not the mesenteric, renal, or femoral arteries. It depletes both peptides in blood vessels that supply the heart and respiratory tract of the guinea pig. Thus, at least in these vascular beds in the rat, a nonsensory role is possible.

Intravenous injection of CGRP into rats causes a marked fall in mean arterial blood pressure and positive chronotropic and inotropic responses. Intradermal injection of CGRP increases blood flow in the skin of rabbits and humans. In vitro, CGRP relaxation of the rat aorta is endothelium-dependent, whereas relaxation of human, rabbit, and feline pial arteries and mesenteric and lingual arteries from the rabbit is endothelium-independent. CGRP (10^{-10} - 10^{-7} M) does not affect the norepinephrine dose-response relation in feline middle cerebral or rabbit central ear arteries. However, vasoconstriction by field stimulation of rabbit central ear arteries is inhibited in a dose-dependent manner by CGRP, suggesting a presynaptic influence as one possible role for this peptide. Evidence for other physiological roles, including a direct neurovasodilator function, awaits further study.

Enkephalins

In 1975, Hughes et al. identified two low-weight peptides from the brain and called them methionine (met)-enkephalin and leucine (leu)-enkephalin. Subsequently, other structurally related compounds were found and together are considered endogenous ligands for opioid receptors. There is some preliminary evidence that these substances should be considered as putative vasodilator transmitters. Enkephalin immunoreactive nerve fibers and cell bodies have been found in the superior cervical, inferior mesenteric, and coeliac-sympathetic ganglia of the guinea pig and rat. The origins and projections of these cell bodies are not established, but they may extend centrally. Enkephalin-like immunoreactivity has been observed in adrenal medullary gland cells of the rat, guinea pig, and cat. These authors propose that adrenal medulla opioid peptides might be released and circulate in the blood stream as hormones that interact with peripheral tissues.

Enkephalins infused into the isolated perfused hind limb of the cat cause vasodilation that is blocked by naloxone and also by diphenhydramine. Thus, enkephalins might be acting on vascular smooth muscle directly and also indirectly by releasing histamine, an effect established at other sites. The possibility that the enkephalins were causing reflex vasodilation via carotid or aortic reflexogenic zones or acting centrally was not excluded. In isolated vascular tissue, including cerebral arteries, specific enkephalin agonists caused vasodilation. The receptors on which they act may have different characteristics from those found in the brain.

In summary, although enkephalins may be involved in neurogenic inhibition of vascular tone, evidence in support of such a possibility is flimsy.

Conclusion

Criteria for transmission have been satisfied at least for acetylcholine, vasoactive intestinal peptide, and dopamine. There is as yet no definitive survey of the sphere of influence of one particular transmitter for any regional bed in any one species. There is good reason to expect that a number of other putative substances included in this review may be found to be neurotransmitters or neuromodulators. Possibly others await discovery. We have only shadowy glimpses of the physiological role of these neural systems. Compared to the sympathetic nervous system, they show considerable variability. In at least one instance, different transmitters seem to subscribe the same role in different species, and the effectiveness of the same system differs in particular members of the same species. If the observations to date are generally correct, they suggest that the parasympathetic nervous system as we know it is composed of a number of different efferent outflow systems, each with different functions and transmitters. The possibility of charting these systems is an exciting prospect.

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J A Bevan and J E Brayden

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